Magnetic Beads based Immunoaffinity Capillary Electrophoresis of Total Serum IgE with Laser-Induced Fluorescence Detection

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Labeling of the secondary antibody. LIF is one of the most sensitive detection modes in CE, and FITC is the most commonly used labeling dye. However, there is some problem using FITC in the reported IA-CE method because acidic buffers need to be used to dissociate the immune complexes. The fluorescence of FITC is only significant at basic pH and decreases dramatically under acidic condition. The Alexa Fluor 488 reactive dye (Figure S1-A) is similar to fluorescein in terms of absorption and emission maxima, respectively around 494 and 519 nm. It has a tetrafluorophenyl ester moiety that reacts efficiently with primary amines of protein to form stable dye-protein conjugate. The produced protein conjugate is known to be brighter and more photostable than the FITC conjugate. Furthermore, there is no

large variation of the fluorescence intensity of Alexa Fluor 488 conjugate in the range going from pH 4 to 10. This has been verified experimentally (S-1B) and further tested down to a pH of 2.2, corresponding to the one of the used elution and separation buffer (10% (v/v) acetic acid). Even if a significant decrease of fluorescence signal is observed for pH below 4, the conjugate remains fluorescent even under rather strong acidic condition. Moreover, taking into account that the proposed method integrates a t-ITP step with the presence of a long plug of LE (pH 7.4), the final pH of the zone in which the secondary labeled antibody will be detected can potentially be higher than 2.2. Consequently, the detection would still be rather sensitive, even if not optimum, under our experimental conditions.

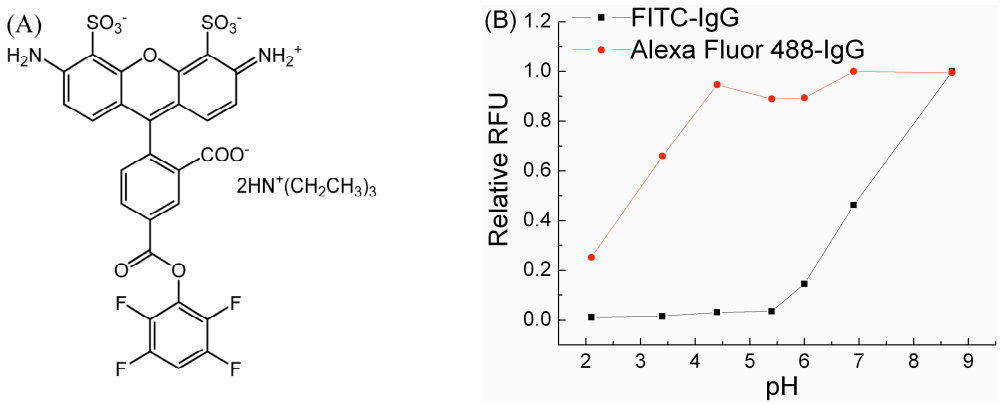

Figure S1. (A) Structure of Alexa Fluor 488 carboxylic acid, bis-triethylammonium salt. (B) Effect of pH on the relative fluorescence intensity of FITC-IgG and Alexa Fluor 488-IgG.